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EGG CAPSULES AND EARLY DEVELOPMENT IN *SIPHONARIA DIEMENENSIS* (QUOY & GAIMARD, 1833) AND *SIPHONARIA BACONI* (REEVE, 1856).

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SUMMARY

Spawn of two species of the littoral pulmonate genus *Siphonaria* from Victoria are described and found to differ only in a few minor features. Development of the two species is also similar and is followed from egg-laying to hatching of a free swimming veliger phase. *S.diemenensis* is in general more vigorous and develops more rapidly than *S.baconi* under the same conditions. Measurements of embryos and capsules at different developmental stages show that while the embryo increases in overall size from egg to hatching, the capsule size does not alter significantly during this period.

INTRODUCTION

Molluscs of the marine pulmonate gastropod genus *Siphonaria* are common members of communities on Victorian rocky shores. *S.diemenensis* occurs throughout the littoral zone on open rock platforms and, to a lesser extent, on sheltered rocks. *S.baconi* has a more restricted distribution, but is also found on open rock platforms in association with *S.diemenensis* (Macpherson & Gabriel, 1962).

The eggs, spawn and early development of a number of littoral gastropods has been described for the Sydney area by Anderson (1960, 1961, 1962, 1966) and for the Melbourne area by Murray (1962, 1966, 1969, 1970) and Black (1976), but these do not include marine pulmonates. The only work of this type on *Siphonaria* is for *S.pectinata* from Florida (Voss, 1959). The aim of the present work is to describe the spawn and follow the early development within the capsule through to hatching; a later extension is planned to study the growth of the veliger from hatching to metamorphosis.

MATERIALS AND METHODS

Spawn from *S.diemenensis* and *S.baconi* was collected from a sandstone reef at Portsea, Victoria during the summer of 1975 - 6. Individual egg masses were measured and kept in litre beakers

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containing glass filtered seawater (pore size 3μm), which was changed weekly, and covered with polythene to reduce bacterial contamination. Adults of each species were also collected and placed in a tidal tank system at La Trobe University; spawn was produced and its subsequent development followed in the tank. All spawn was maintained at 15°C, and a daily sample examined under the microscope. Photographs were taken using an Olympus PM-6 camera mounted on a GB microscope.

DESCRIPTION OF SPAWN

Siphonaria diemenensis

The eggs are laid as a ribbon in a collar or spiral-shaped mass usually almost circular in outline (Fig.1A). Mean dimensions are given in Table 1. Each egg is contained in a capsule and the capsules are connected by threads (Fig.2). The network of capsules is embedded in a fairly turgid clear jelly surrounded by a clear protective covering layer 0.34mm thick. The colour of the spawn varied with stage of development, from a pale or mid-yellow colour at the time of deposition to the mid-veliger stage, and a darker golden yellow from late mid-veliger stage to hatching.

TABLE 1 : Mean dimensions of the egg masses of two *Siphonaria* species. (S.D. - standard deviation)

	S. diemenensis	*S. baconi*
Max. dimensions mm	10.31 x 11.26 (S.D.2.5) (S.D.2.7)	9.19 x 7.80 (S.D.6.7) (S.D.5.2)
Ribbon width mm	1.19 (S.D.1.1)	2.83 (S.D.0.4)
Ribbon height mm	5.31 (S.D.2.0)	1.64 (S.D.0.9)

Siphonaria baconi

The eggs are also laid as a ribbon (Fig.1C), which is again usually collar shaped (although never a spiral), but frequently irregular in outline; occasionally a very wide open collar is laid, or a long irregular trailing ribbon. The collars are without exception much flatter than those of *S.diemenensis.* Mean dimensions are given in Table 1. The capsules each contain one egg and are similar in shape and size to those of *S.diemenensis,* but the jelly is less turgid and its clear covering varies in thickness from 0.18mm at the sides to negligible thickness on top. Sand grains, diatoms and other debris characteristically adhere to the jelly masses from Portsea, although were not available for adherence to masses laid in the tidal tanks. The spawn colour differs from *S.diemenensis,* varying from pale cream to white at deposition to trochophore stage, cream at trochophore to mid-veliger and dark cream in the advanced veliger stage to hatching.

Spawning time

Initial limited observations suggest that new spawn is only deposited by *S.diemenensis* during spring tides. The periods when spawn was laid in laboratory tanks also coincided only with spring tides. In contrast, newly deposited spawn of *S.baconi* was collected on the shore and laid in laboratory tanks only during neap tides. However, these preliminary observations require further verification in the field.

EARLY DEVELOPMENT

Siphonaria diemenensis

Egg masses spawned in captivity and maintained in tidal tanks at 15°C reached hatching stage in 7 to 10 days. Hatching was determined as the date when the first free swimming veliger larvae were released. These limits may be more variable in the natural habitat where masses are exposed to greater fluctuations in environmental conditions.

A series of developmental stages is described below and illustrated in Fig.3 A to I. Maximum dimension of early stages and maximum shell length of later stages are given in Table 2. Cleavage

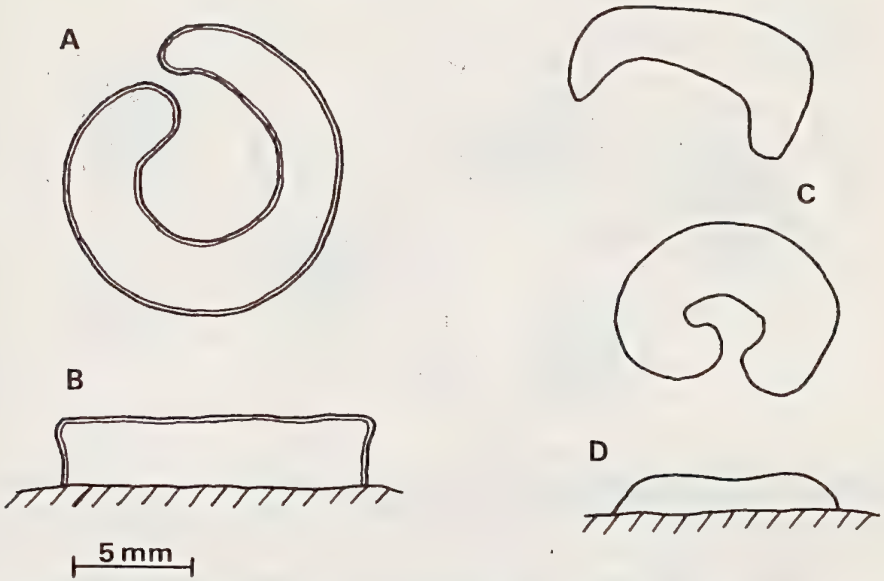

FIGURE 1. A, *S.diemenensis* : View from above of typical egg mass. B, *S.diemenensis* : Lateral view of egg mass attached to substrate; capsules are embedded in a turgid jelly with a distinct clear layer over the surface of the mass. C, *S.baconi* : View from above of two egg masses: there is more variation in shape in this species. D, *S.baconi* : Lateral view of egg mass attached to substrate; capsules are embedded in a less turgid jelly than *S.diemenensis* and there is no distinct clear layer over the surface of the mass.

occurs rapidly once the eggs are laid and within 24 hours a placula blastula of large distinct cells is formed (Fig.3 A,B,C.). Macromere diameter decreases from 50μm in the 2-cell stage to 29μm in the early blastula stage but thereafter cells were too small for accurate measurements. These cells further subdivide during the next 24 hours. One surface of the blastula becomes concave (Fig.1 E) and in the centre of this area cells move into a slit-like blastopore as gastrulation occurs (Fig.1 D). Large yolky macromeres are visible inside the early blastula, but become obscured by the time gastrulation is completed 2 days after laying. During the third day a band of very small cilia develop (length 4 μm) and the embryo, still gastrula-shaped but now termed a trochophore, begins to move slowly (Fig.1 E). Polar bodies extruded during early cleavage remain visible up to the trochophore stage in some specimens.

During the fourth day a thin triangular shell rudiment (max. length 73μm) develops enclosing undifferentiated tissue (including much yolk) which forms the visceral hump. Velar lobes start to grow out and the cilia (length 10 μm) enlarge to form the velum. The foot begins to develop as a short square outgrowth beneath the velum and a very thin operculum becomes visible on its posterior surface. This is the early veliger stage (Fig.1 G). Much growth of shell, velum and foot and differentiation of internal organs occurs over the next 3 to 4 days to form ultimately the advanced veliger stage which is ready to hatch. By the mid-veliger stage (Fig.1 H) a pair of prominent statocysts have formed and the stomach and digestive gland are distinct. The larval heart has begun to beat spasmodically. Cilia have formed over the surface of the foot, and the operculum now projects beyond the foot edge. Torsion occurs and can be easily recognized by the appearance of the rectum in a dorsal position above the velum, with the anus opening into the left side of the mantle cavity. Shell growth continues asymmetrically due to torsion and the thickened glandular mantle edge is attached to the shell rim. By the seventh to tenth day after laying torsion is complete and the

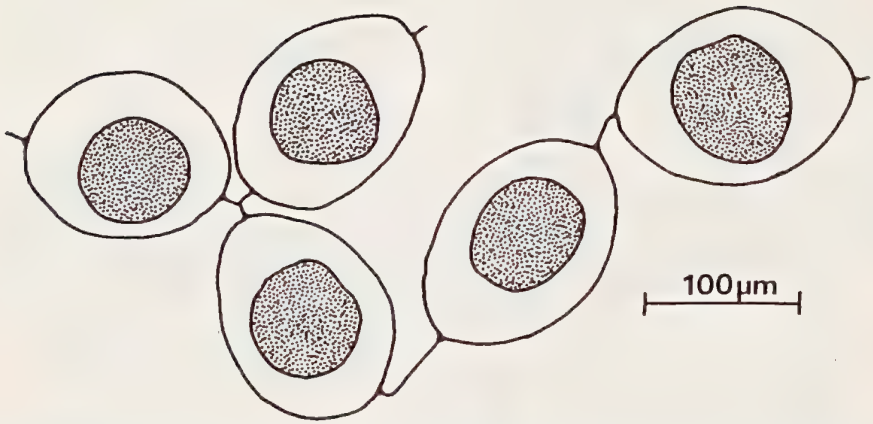

FIGURE 2. *S.diemenensis* : Detail of capsules (containing embryos) which, in the spawn, are embedded in jelly; they are connected into strings by tough threads and sometimes one capsule is connected to two others.

TABLE 2 : Dimensions of encapsulated developmental stages in *S.diemenensis*

Stage	Mean µm	95% Confidence Limit	Standard Deviation	90% Confidence Limits
Uncleaved egg - max. dimension	86	± 1.28	2.7	1.4 → 4.9
Two cell " "	112	± 1.05	2.2	1.6 → 5.4
Four cell " "	88	± 5.48	6.6	5.9 → 130.6
Eight cell " "	94	± 3.03	4.5	3.6 → 27.5
Sixteen cell " "	101	± 3.02	6.3	5.0 → 19.1
Blastula " "	103	± 1.36	6.1	5.4 → 9.6
Gastrula " "	100	± 0.98	4.3	3.8 → 6.9
Trochophore " "	95	± 1.03	4.6	4.0 → 7.2
Early veliger " "	107	± 0.91	4.1	3.3 → 6.0
Mid-veliger - max. shell length	111	± 3.61	13.3	12.9 → 27.1
Advanced veliger " "	132	± 2.61	10.1	9.2 → 17.6

mantle edge has become detached from the shell allowing velum and foot to be retracted into the shell, at first partially and then completely (Fig.1 I). The lumina of digestive gland, stomach and style sac have opened in preparation for feeding and a few remaining yolky spheres from the digestive gland are often visible in the stomach as they are rotated by the cilia. This marks the end of encapsulated development.

Veligers in the outermost layers of jelly develop most rapidly and constantly rotate inside their capsules by vigorous movements of the velar cilia. Escape from the capsule appears to be facilitated by a softening of the wall which is split open with the velar cilia. Veligers continue to escape until the mass is completely spent, which may be up to a week after the onset of hatching.

Capsule measurements were taken at each developmental stage for a number of egg masses with capsule size expressed as capsule length:width ratio in µm (Table 3). Samples of capsules from

different parts of the same egg mass were measured and found to be of similar size as no significant differences were found using Duncan's Multiple Range Test (Steel and Torrie, 1960). Similarly, results of Duncan's Test performed on ratios of capsules from different masses (at the same developmental stage) indicate that capsule size does not differ significantly at the 0.1% protection level from mass to mass. Capsule ratios compared from egg laying through to hatching using the same test were also shown not to differ significantly.

TABLE 3 : Dimensions of capsules containing mid-veligers in *S.diemenensis* and *S.baconi*

	S.diemenensis		*S baconi*	
	Mean µm	Standard Deviation	Mean µm	Standard Deviation
Capsule length	159	5.59	206	9.41
	(Range 144 – 172)		(Range 180 – 240)	
Capsule width	125	4.91	156	5.71
	(Range 116 – 136)		(Range 144 – 188)	
Ratio length:width	1.3	0.05	1.3	0.07

Siphonaria baconi

Egg masses raised under the same conditions as those of *S.diemenensis* took longer to develop, from 13 to 16 days. However, masses of this species were found to be more difficult to rear in captivity when collected from the shore after deposition, suggesting that they may be more specific in their ecological requirements. This may be one reason for the much greater success of *S.diemenensis* on most Victorian rocky shores.

TABLE 4 : Mean dimensions of encapsulated developmental stages in *S.baconi*

Stage	Mean µm	95% Confidence Limit	Standard Deviation	90% Confidence Limits
Uncleaved egg - max. dimension	96	± 0.96	2.0	1.3 → 4.9
Two cell " "	120	± 1.71	3.6	2.5 → 9.6
Four cell " "	125	± 1.85	3.9	3.1 → 11.4
Blastula " "	119	± 1.67	7.7	6.8 → 13.1
Gastrula " "	111	± 1.0	4.5	3.9 → 7.4
Trochophore " "	110	± 0.8	3.1	2.4 → 5.9
Mid-veliger - max. shell length	108	± 2.0	4.1	3.3 → 12.1
Advanced veliger " "	149	± 3.1	6.7	5.7 → 21.7

Development follows the same course as in *S.diemenensis* except for the longer time taken to reach hatching. Dimensions of the various stages are given in Table 4, and again they do not differ significantly from those of *S.diemenensis* (Duncan's Test). Capsule dimensions for *S.baconi* are given in Table 3. Like *S.diemenensis*, capsule ratio does not vary significantly in different parts of the same egg mass, nor from egg laying through to hatching. The mean capsule ratios of *S.baconi* and *S.diemenensis* (at the same developmental stage) do not differ (1:3 for each), although variation in ratios is greater for *S.baconi* (as shown by a standard deviation of 0.07 as against 0.05). Results of Duncan's Test indicate that this difference is not significant.

DISCUSSION

Spawn of *S.diemenensis* and *S.baconi* can be most easily distinguished by size and shape. The spawn ribbon of *S.diemenensis* is of greater height (5.31 mm) and is more uniform in shape (either a horseshoe or a spiral) than that of *S.baconi* which is flatter (1.64 mm) and often irregular in

outline with foreign matter adherent. Voss (1959) has shown that *S.pectinata* from Florida produces spawn in the form of small oval ribbons which are similar in colour and dimensions to those of *S.diemenensis* and *S.baconi* and intermediate between them in height (2 mm). Members of the Family Siphonariidae, therefore, produce jelly masses in ribbons resembling most diotocardian and primitive monotocardian prosobranchs and more primitive opisthobranchs (Anderson, 1960; Fretter and Graham, 1962). Like *S.pectinata* (Voss, 1959), the spawn of *S.diemenensis* and *S.baconi* contains a mass of egg-shaped capsules embedded in a jelly matrix and enclosed by a toughened wall. The capsules of all three species are of similar dimensions when laid and are connected into strands by a fine thread from each end. It is interesting to note that larger egg capsules of almost identical structure are produced by the pyramidellid *Odostomia* (Fretter and Graham, 1962), a prosobranch quite unrelated to *Siphonaria*. Egg-shaped capsules are also produced by members of the opisthobranch genus *Doto* but they are smaller when laid than those of *Siphonaria* and are not connected by threads (Kress, 1975). Kress (1975) also reported occasional twins for *Doto*, but these have not been observed in *Siphonaria*.

Voss (1959) quotes from Dieuzeide (1935) that development time for *S.pectinata* in Algeria is 20 days at 57 - 75°F but the present results show that development time for the two Victorian species is more rapid (10 to 16 days) at a predominantly lower temperature (15°C, 58°F). The course of development for *S.pectinata* is not described by Voss (1959), but for *S.diemenensis* and *S.baconi* is very similar. Both species lay large numbers of small eggs which hatch into well developed veligers. The gut and other organs of these larvae are highly differentiated, torsion is complete, most of the yolk has already been utilized and the large velar cilia are obviously adapted for a long planktotrophic life. In this respect they resemble the veligers of many monotocardian prosobranchs (Lebour, 1937, 1945; Fretter and Graham, 1962; Fretter and Pilkington, 1970) which may spend up to two months or more in the plankton. Encapsulated embryonic development is similar to that described for the Australian prosobranchs *Bembicium nanum* and *B.auratum* by Anderson (1961, 1962), although *Bembicium* lacks a distinct capsule and Anderson did not record detailed embryonic measurements. Kress (1975) found for the British opisthobranch *Doto* that capsule length to width ratio decreased during development, but this did not occur in *Siphonaria*. However, the length to width ratio of capsules at the same developmental stage was found to be constant for all three *Doto* species, as it is for the two *Siphonaria* species.

Advanced veligers of *S.diemenensis* and *S.baconi* hatch in a similar manner to *S.pectinata* (Voss, 1959). Hatching in the Australian species is also probably stimulated by wetting of the egg mass as Voss found for *S.pectinata;* she suggests that this may be a natural stimulant for ribbons laid above low water.

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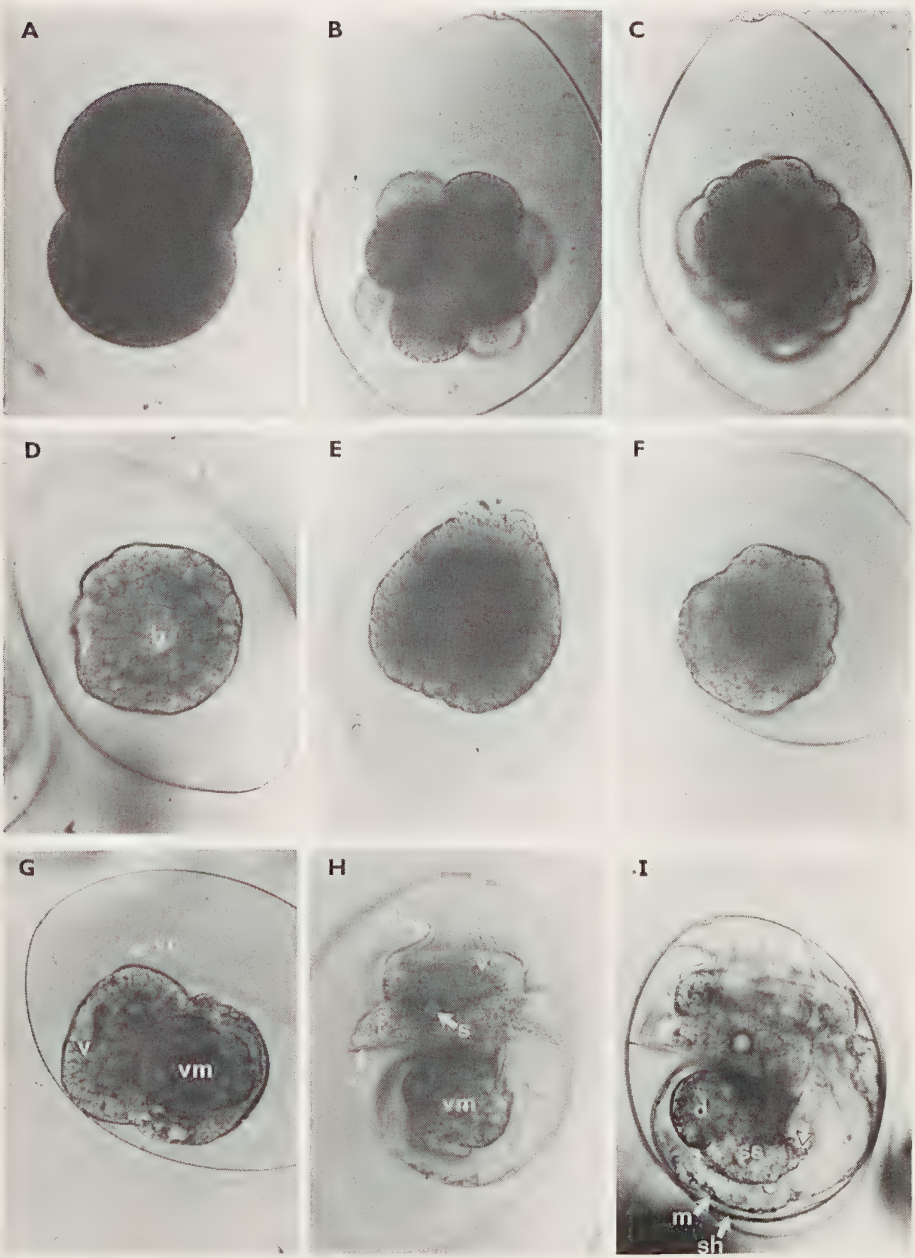
FIGURE 3. Series of developmental stages in *S.diemenensis*. Magnification x730.
A, Two cell stage; 2 hours after deposition. B, Eight cell stage; 6 hours after depositon. C, Early blastula; 21 hours after deposition. D, Late blastula showing blastopore (b); 28 hours after deposition. E, Gastrula; 2 days after deposition. F, Trochophore (cilia not visible, but present); 3 days after deposition. G, Early veliger with velum (v) and velar cilia (vc), undifferentiated visceral mass (vm), shell rudiment (sr) (foot not in focus); 4 days after deposition. H, Mid-veliger undergoing torsion showing visceral mass (vm) differentiating, statocyst (s), larger shell (sh) with mantle edge (m) still attached, foot (f) with cilia and projecting operculum (o); 5 days after deposition. I, Advanced veliger, ready to hatch showing stomach with style sac (ss), digestive gland (d), rectum (r), the shell (sh) now filling the capsule and the mantle edge (m) which has detached allowing the veliger to completely retract; 10 days after deposition.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